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Identification and characterization of a pancreatic intrinsic factor in the dog

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BATT, ROGER M., NEIL U. HORADAGODA, LYNN McLEAN, DAVID B. MORTON, AND KENNETH W. SIMPSON. *Identification and characterization of a pancreatic intrinsic factor in the dog.* Am. J. Physiol. 256 (Gastrointest. Liver Physiol. 19): G517–G523, 1989.—An intrinsic factor has been identified in the canine pancreas, and output and properties of this protein have been compared with those of gastric intrinsic factor in the dog. Mean concentrations of intrinsic factor and peak outputs per minute were approximately 5- to 10-fold higher in pure pancreatic juice after stimulation with secretin and cholecystokinin, respectively, than in pentagastrin-stimulated gastric juice. Purified gastric and pancreatic intrinsic factors had an identical molecular mass of 65 kDa, estimated by gel filtration on Sephacryl S-200, while sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated single bands corresponding to 53 kDa. Immunoblots showed that rabbit polyclonal antiserum to canine gastric intrinsic factor cross-reacted with canine pancreatic intrinsic factor. Gastric and pancreatic intrinsic factor-cyano[^{57}Co]cobalamin complexes exhibited comparable association constants for ileal receptors in canine brush-border vesicles, while there was minimal binding to jejunal vesicles. These findings demonstrate that the canine pancreas is an important source of an intrinsic factor that closely resembles gastric intrinsic factor in the dog.

absorption; cobalamin; small intestine; stomach

MANY MAMMALIAN species have developed a complex process that facilitates the selective absorption of cobalamin (Cbl) and rejection of potentially harmful Cbl analogues by the small intestine (12). This process involves binding of Cbl to intrinsic factor (IF), a glycoprotein secreted by the stomach, and subsequent attachment of this IF-Cbl complex to specific receptors in the microvillar membrane of ileal enterocytes.

Early reports suggested that the canine stomach does not secrete IF and that physiological absorption of Cbl is not mediated by IF in this species (36). Subsequently, a canine gastric IF has been identified (24), although this protein appears to represent only a small proportion of the total Cbl-binding capacity of gastric mucosa and of nonstimulated gastric juice. Binding of IF-Cbl complexes to IF receptor protein in ileal mucosa has also been demonstrated (23, 24), and the receptor has been purified (30), providing further evidence that IF could play a physiological role in the absorption of Cbl in the dog. In addition, lysosomal localization during passage through the ileal enterocytes strongly supports absorption of Cbl by receptor-mediated endocytosis (14), consistent with the need for prior binding of Cbl to IF as a ligand.

Nevertheless, it is not clear whether IF is exclusively of gastric origin in the dog or whether an alternative source of IF might exist. Indeed, the finding that experimental gastrectomy has no effect on Cbl absorption indicates that the canine stomach is not essential for the normal absorption of Cbl in the dog (1, 2). Furthermore, there is some evidence that the pancreas could be particularly important, since ligation of pancreatic ducts, either alone or in combination with gastrectomy, appears to interfere with Cbl absorption (2) and low serum Cbl concentrations have been reported in dogs with naturally occurring exocrine pancreatic insufficiency (7). These findings could reflect the presence of a pancreatic IF, but an alternative role for the canine pancreas in the absorption of Cbl cannot be excluded. For example, it has been suggested that malabsorption of Cbl in human patients with exocrine pancreatic insufficiency may be due to defective degradation of R-binders, which are non-IF Cbl-binding proteins (5). In addition, while Cbl-binding capacity is relatively low and no IF has been identified (10, 11), pure human pancreatic juice has been shown to have a direct effect on the uptake of Cbl by the guinea pig ileum (11). However, since exocrine pancreatic insufficiency rarely appears to result in Cbl deficiency in humans (10), it is possible that the pancreas could play a more fundamental role in the handling of Cbl in the dog.

The present study compares the Cbl-binding capacities of stimulated gastric and pancreatic secretions and has identified the pancreas as an important source of IF in the dog. A preliminary report of these findings has been published (15).

MATERIALS AND METHODS

Collection of gastric and pancreatic secretions. Stimulated secretions from the stomach and pancreas were collected from six clinically healthy adult greyhounds (5 male, 1 female; 25–34 kg body wt) maintained on a diet of Pedigree Chum and Chum mixer (Pedigree Petfoods, Melton Mowbray, UK).

After an overnight fast, animals were sedated with 0.2 mg/kg acetylpromazine maleate (Berkace, Berk Pharmaceuticals, Eastbourne, UK) given intramuscularly and

were then placed in left lateral recumbency. Gastric juice was collected through an aspiration tube (3-mm internal diameter, 125-cm length) positioned by use of radiography with the tip in the most dependent part of the stomach. Resting gastric contents were aspirated for 10 min, and then the stomach was rinsed with 50 ml warm sterile isosmotic saline. Basal secretions were aspirated for 10 min. Stimulated secretions were then collected on ice for six consecutive 10-min periods after intramuscular injection of pentagastrin (Peptavlon, Imperial Chemical Industries, Macclesfield, UK) at a dose of 6 μg/kg. Immediately after collection, mucus was removed by centrifugation at 5,000 *g* for 15 min at 4°C in a Sorvall RC5B centrifuge (Du Pont, Hertfordshire, UK). The volume of gastric juice in each 10-min sample was recorded, and the pH was measured by use of a Pye Unicam pH meter (Pye Unicam, Cambridge, UK). Gastric acid output was estimated by titrating 1-ml aliquots of each sample to pH 7.0 using 0.01 M sodium hydroxide. Gastric juice was then depepsinized by increasing the pH to 10.0 with 1 M NaOH and, 20 min later, adjusting to pH 7.0 with 1 M HCl. Samples were stored at −20°C before further assay.

Pancreatic juice was collected by cannulation of the main pancreatic duct at laparotomy. After an overnight fast, anesthesia was induced using intravenous thiopentone sodium (Intraval sodium, May and Baker, Dagenham, UK) at a dosage of 10–15 mg/kg, and maintained with a halothane-nitrous oxide-oxygen mixture administered by a ventilator. The pancreas was exteriorized, and then the major pancreatic duct was isolated by blunt dissection and ligated with braided polyamide (Nurulon 2/0, Ethicon) close to its point of entry into the duodenum. The duct was incised proximally and cannulated with an 8-gauge infant feeding catheter (Vygon UK, Cirencester, UK), which was secured with a second ligature placed around the duct.

Pancreatic juice was collected on ice for a total of 30 min during hormonal stimulation. Secretin (Boots, Nottingham, UK) was administered intravenously at a dosage of 1 Crick-Harper-Raper unit (0.25 clinical units)/kg and stimulated pancreatic juice was collected for 1-min intervals over 10 min followed by a 5-min collection. Immediately after this collection, cholecystokinin (CCK, Boots) was injected intravenously at a dosage of 1 Crick-Harper-Raper unit (1 clinical unit)/kg, and the stimulated pancreatic secretions were collected as described for secretin. Pancreatic juice was stored at −20°C before further assay.

Chemicals. Polyacrylamide, sodium dodecyl sulfate (SDS), and *N′,N′* methylene-bis-acrylamide were supplied by BDH (Dagenham, UK). Ammonium persulfate, *N,N,N′,N′*-tetramethylethylenediamine, bromophenol blue, Coomassie brilliant blue, and high molecular mass standards for electrophoresis were obtained from Bio-Rad Laboratories (Watford, UK), and nitrocellulose membranes (0.45-μm pore size) were from Pharmacia (Milton Keynes, UK). Goat anti-rabbit alkaline phosphatase conjugate, *o*-dianisidine, and β-naphthyl acid phosphate were from Sigma Chemical (Poole, UK). Cyano[^{57}Co]Cbl (sp act 100–300 μCi/μg) was supplied by Amersham International (Amersham, UK).

Purification of gastric and pancreatic intrinsic factors. Gastric mucosal extracts were prepared from the fundic and pyloric regions of stomachs from clinically healthy adult crossbred dogs, and gastric IF was purified by ammonium sulfate fractionation, followed by affinity chromatography on a Cbl affinity matrix as described in detail previously (13). Pancreatic IF was purified from canine pancreatic juice, collected as described above, by a procedure identical to that detailed for the purification of gastric IF after ammonium sulfate fractionation (13). The purified proteins were assayed for Cbl-binding capacity and then stored at −20°C either unbound or bound to cyano[^{57}Co]Cbl.

Characterization of gastric and pancreatic intrinsic factors. The molecular masses of purified gastric and pancreatic IFs bound to cyano[^{57}Co]Cbl were estimated by gel filtration on Sephacryl S-200 superfine (Pharmacia) in a column (90 cm × 3.8 cm^2) calibrated by use of dextran blue, bovine serum albumin, ovalbumin, and carbonic anhydrase (Sigma). The column was equilibrated and eluted with 0.1 M phosphate-buffered saline (PBS) pH 7.4, and the elution profiles for gastric and pancreatic IFs were determined by assay of radioactivity.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of purified gastric and pancreatic IFs was performed using a discontinuous system (19) with a 5–15% polyacrylamide gradient. Both purified proteins were first concentrated by ultrafiltration (Minicon-B 15 Concentrator, Amicon, Stonehouse, UK), then ~50 μg of each was suspended in an equal volume of 0.12 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 6.8, containing 10% (wt/vol) SDS, 20% (vol/vol) glycerol, and 0.1% (wt/vol) bromophenol blue and boiled for 4 min. After electrophoresis, gels were stained with Coomassie brilliant blue and scanned on a Bio-Rad model 620 Video Densitometer. Molecular mass was calculated by regression analysis of relative mobilities of the high molecular mass standards included in each gel. The results were unaffected by addition of mercaptoethanol (2.5% vol/vol) to the buffered SDS solution prior to electrophoresis.

Polyclonal antibodies to canine gastric IF were raised in New Zealand White rabbits by immunization with the purified protein (0.5 mg/ml in PBS), emulsified in an equal volume of Freund's complete adjuvant (Sigma). A total of 2.5 mg of antigen was injected at four subcutaneous sites at intervals of 2 wk, and antiserum was harvested 7 days after the fifth injection. Cross-reactivity with pancreatic IF was examined after SDS-PAGE of purified gastric and pancreatic IFs by electrophoretically transferring unstained proteins to nitrocellulose membranes (32) using an LKB Multiphor II Nova Blot system. The nitrocellulose sheets were incubated at room temperature for 24 h in PBS containing 0.05% Tween-28 (Sigma) and the rabbit antiserum (1:64,000) to purified gastric IF. Bands were visualized by incubation with goat anti-rabbit alkaline phosphatase conjugate at room temperature for 2 h and subsequent incubation with color reagent comprising 0.6 M sodium borate, pH 9.7, *o*-dianisidine, and β-naphthyl acid phosphate.

Brush-border vesicles were prepared from the jejunal and ileal mucosa of a clinically healthy adult crossbred dog as described previously (17). Binding of gastric and pancreatic IF-cyano[^{57}Co]Cbl complexes was determined essentially as described previously (8) by incubation of these vesicles at room temperature in a medium comprising (in mM) 300 mannitol, 2 $MgCl_2$, 1 $CaCl_2$, 20 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid adjusted to pH 7.4 with Tris, and 550 pg/ml cyano[^{57}Co]Cbl as IF-Cbl complexes. At timed intervals, 0.1-ml aliquots were removed and diluted 20-fold in a stop buffer consisting of incubation medium without cyano[^{57}Co]Cbl at 4°C. Each diluted aliquot was filtered on a 0.45-μm filter (Millipore, Harrow, UK), washed with 25 ml stop buffer, and assayed for radioactivity. Association constants for gastric and pancreatic IFs were determined by incubation of brush-border vesicles for 45 min at 20°C in medium containing between 12.5 and 700 pg/ml cyano[^{57}Co]Cbl as IF-Cbl complexes and by subsequent analysis of Rosenthal plots by computer (25). Reversibility of binding was assessed by incubation at room temperature in stop buffer containing 0.3 M EDTA.

Assays. The total and IF Cbl-binding capacities of gastric and pancreatic secretions were assayed by the cobinamide method essentially as described previously (9). Aliquots (100 μl) of suitably diluted sample were delivered into two duplicate sets of tubes each containing 3 ml PBS and 0.1% (wt/vol) bovine serum albumin. To one set of tubes 100 μl PBS were added, and to the other set 100 μl (40 ng) cobinamide. After incubation at 37°C for 30 min, 100 μl cyano[^{57}Co]Cbl were added to all tubes, which were then incubated for a further 30 min at 37°C. Free cyano[^{57}Co]Cbl was separated by adding 250 μl albumin-coated charcoal and centrifuging at 1,000 *g* for 30 min. IF and total Cbl-binding capacities were determined by assay of bound radioactivity in the supernatant of tubes with and without cobinamide, respectively, and nonintrinsic factor (R-binder) Cbl-binding capacities were calculated by difference.

Protein was assayed by the method of Schacterle and Pollack (28) with bovine serum albumin (Armour Pharmaceutical, Eastbourne, UK) as standard. Trypsinogen (EC 3.4.21.4) was assayed with benzoyl arginine ethyl ester (Sigma) as substrate (29) after activation of pancreatic juice at 30°C with porcine enteropeptidase (Sigma) at a final concentration of 0.32 U/ml.

Statistical analyses were performed by use of Student's paired *t* test.

RESULTS

Output of gastric and pancreatic intrinsic factors. Figure 1 shows the outputs of IF, acid, and fluid in gastric juice of five dogs after pentagastrin stimulation. The sixth dog had duodenal reflux during the collection period, therefore the results were not included. Mean gastric IF output peaked between 20 and 30 min after pentagastrin injection and then declined to plateau at a rate approximately three times that of the first 10-min period. The profile for nonintrinsic factor (R-binder) Cbl-binding proteins (not shown) essentially paralleled that of IF. While there were some similarities in the mean outputs of IF and acid, these profiles were not identical and reflected apparent dissociation between outputs of IF and acid in individual animals. Compared with IF and acid, fluid output showed a less marked increase during the first two collection periods and subsequently declined more slowly.

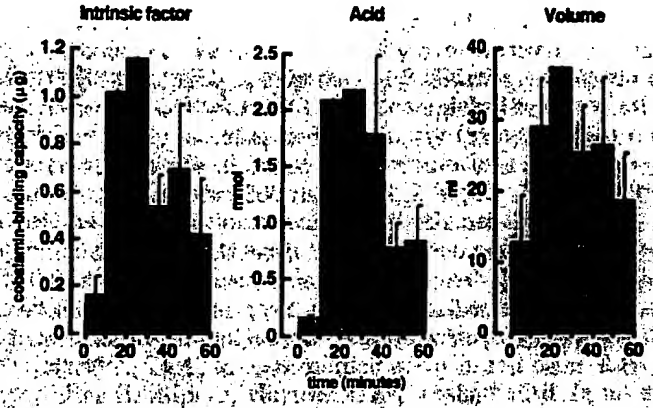

FIG. 1. Outputs of intrinsic factor, acid, and fluid in gastric juice after intramuscular injection of pentagastrin. Data (means ± SE) from 5 dogs are expressed as outputs per 10 min.

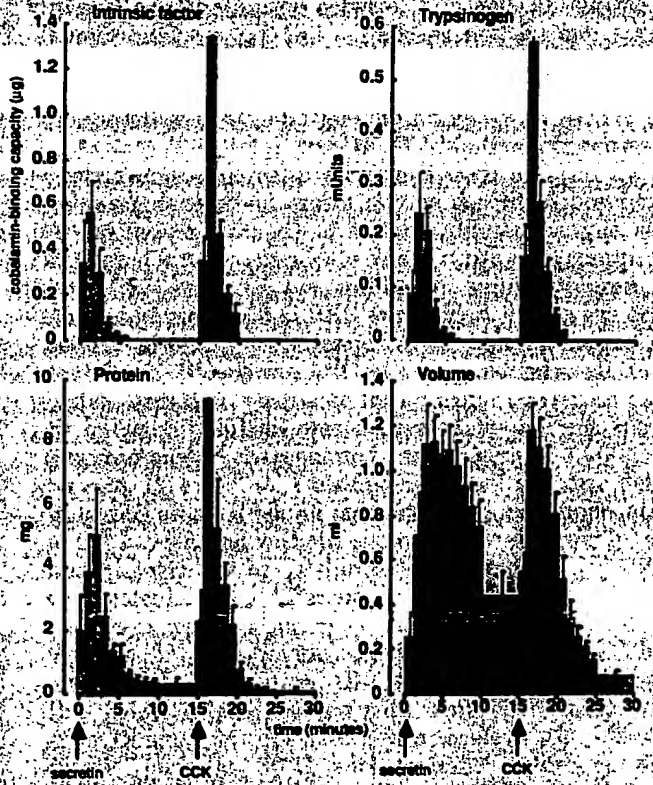

FIG. 2. Outputs of intrinsic factor, trypsinogen, protein, and fluid in pancreatic juice after intravenous injection (arrows) of secretin and cholecystokinin. Data (means ± SE) from 6 dogs are expressed as outputs per minute.

The outputs of IF, trypsinogen, protein, and fluid in pancreatic juice obtained from six dogs after stimulation with secretin and CCK are shown in Fig. 2. Intravenous secretin resulted in some increase in the outputs of IF, trypsinogen, and protein, all of which had fallen markedly within 5 min of injection. In contrast, the rate of

fluid secretion increased considerably to plateau at 3 min after secretin administration and declined gradually during the latter part of the 15-min collection period. While the shapes of the profiles were similar, Fig. 2 shows that mean peak outputs of IF, trypsinogen, and protein were higher after CCK than secretin. In contrast, fluid output peaked earlier and fell relatively rapidly after CCK compared with secretin. The profile of R-binder output from the pancreas (not shown) paralleled that of IF, except that the CCK and secretin peaks were comparable in height.

Table 1 compares the stimulated outputs of IF and R-binders from the stomach and pancreas. IF represented ~50% of the total Cbl-binding capacities of both gastric and pancreatic secretions. However, the proportion of IF in pancreatic juice was greater after CCK than secretin, since the former resulted in a significantly enhanced output of IF but no. of R-binders compared with secretin. Mean concentrations and peak outputs of IF per minute were approximately 5- to 10-fold higher in pancreatic juice after stimulation with secretin and CCK, respectively, than in pentagastrin-stimulated gastric secretions.

Characterization of gastric and pancreatic intrinsic factors. Purification of gastric IF by affinity chromatography resulted in up to an 80-fold increase in specific activity compared with the ammonium sulfate extract of gastric mucosa, and cobinamide inhibition of R binders demonstrated that 90–98% of the Cbl-binding capacity of the purified protein was due to IF. Affinity chromatography of pancreatic juice resulted in up to a 700-fold increase in specific activity of pancreatic IF, and cobinamide inhibition showed that pancreatic IF represented ~98% of the Cbl-binding capacity of the purified protein.

Gel filtration of purified gastric IF bound to cyano[^{57}Co]Cbl yielded a single symmetrical peak with an apparent molecular mass of 65 kDa. Purified pancreatic IF bound to cyanol[^{57}Co]Cbl yielded an elution profile and hence an apparent molecular mass identical to that of gastric IF. SDS-PAGE of purified gastric and pancreatic IFs revealed a single band for each, corresponding to a molecular mass of ~53 kDa. This is illustrated in Fig. 3, which shows Coomassie blue staining of purified pancreatic IF and immunoblots of both purified gastric and pancreatic IFs, all of which were run in parallel on SDS-PAGE. These immunoblots also demonstrated that the antiserum to canine gastric IF cross-reacted with canine pancreatic IF.

Figure 4 shows time courses for the binding of purified gastric and pancreatic IF-cyano[^{57}Co]Cbl complexes to brush-border vesicles prepared from canine jejunum and ileum. At each time point, both complexes exhibited significantly greater binding to ileal than to jejunal vesicles ($P < 0.001$), and at 60 min there was an ~10-fold difference between vesicles from these two sites. Association constants for Cbl binding to receptors in ileal vesicles were 2.6 and 2.1×10^{-10} M for gastric and pancreatic IF-Cbl complexes, respectively, binding data

TABLE 1. *Total and peak stimulated outputs of intrinsic factor and nonintrinsic factor (R-binder) cobalamin-binding proteins in gastric and pancreatic secretions*

Stimulation	Gastric Secretion	Pancreatic Secretion	
	Pentagastrin	Secretin	Cholecystokinin
Total output, μg	per 60 min	per 15 min	per 15 min
Intrinsic factor	3.95±1.2	1.35±0.4	2.52±0.2
		($P < 0.05$)	
R-binders	3.55±1.5	1.25±0.4	1.6±0.6
		(NS)	
Concentration in total output, μg/ml			
Intrinsic factor	0.034±0.01	0.13±0.04	0.41±0.03
		($P = 0.001$)	
R-binders	0.024±0.01	0.11±0.04	0.24±0.08
		(NS)	
Peak output, μg/min			
Intrinsic factor	0.13±0.043	0.655±0.16	1.35±0.18
		($P < 0.05$)	
R-binders	0.14±0.06	0.56±0.18	0.64±0.18
		(NS)	

Values for cobalamin-binding capacities for gastric ($n = 5$) and pancreatic secretions ($n = 6$) are means ± SE with significance of differences between secretin and cholecystokinin in parentheses. NS, not significant ($P > 0.05$).

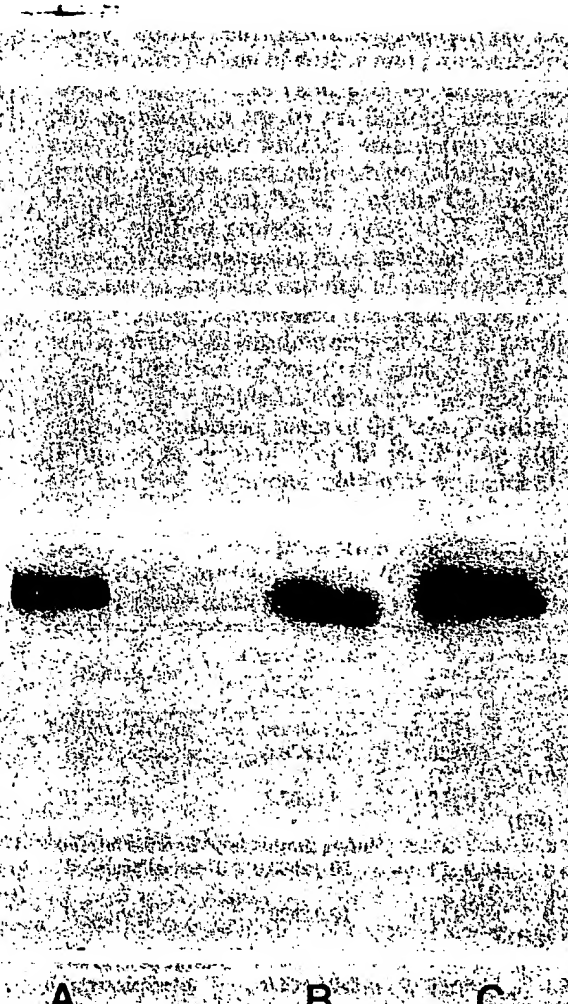

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of preparations run in parallel showing (*A*) Coomassie blue staining of purified pancreatic intrinsic factor and immunoblots of purified (*B*) pancreatic and (*C*) gastric intrinsic factors after incubation with antiserum to gastric intrinsic factor. Positions of high molecular mass standards are indicated in kilodaltons.

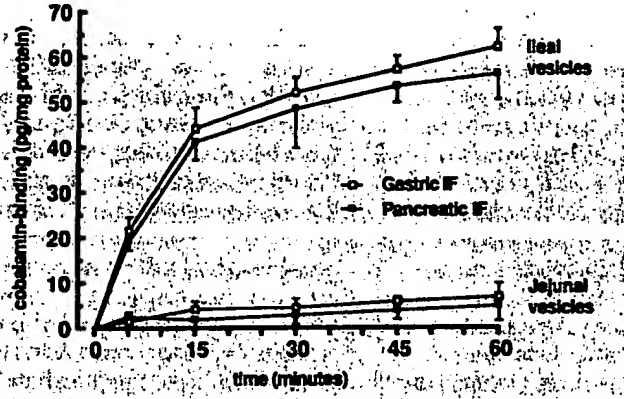

FIG. 4. Time course for binding of purified gastric and pancreatic intrinsic factor (IF)-cyano[^{57}Co]cobalamin complexes to jejunal and ileal vesicles. Data from 6 determinations are expressed as means ± SE.

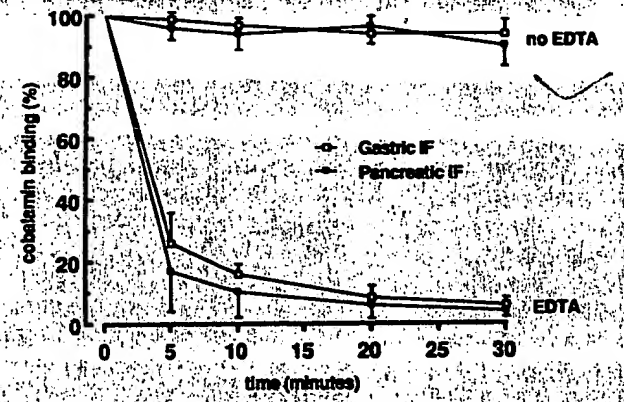

FIG. 5. Effect of EDTA on binding of purified gastric and pancreatic intrinsic factor (IF)-cyano[^{57}Co]cobalamin complexes to jejunal and ileal vesicles. Data from 3 determinations are expressed as means ± SE.

in both cases being compatible with a single receptor. The effects of Ca^{2+} and Mg^{2+} on the binding of the gastric and pancreatic IF-cyano[^{57}Co]Cbl complexes to ileal vesicles are shown in Fig. 5. Vesicles incubated in the stop-buffer solution containing these ions retained >90% of the bound cyano[^{57}Co]Cbl at 30 min. Chelation of these ions with EDTA resulted in markedly enhanced dissociation of cyano[^{57}Co]Cbl from the vesicles and <10% was retained at 30 min.

DISCUSSION

Intrinsic factor (IF) and nonintrinsic factor (R-binder) gastrointestinal Cbl-binding proteins have been characterized previously in the dog (24). IF was identified in pyloric mucosal extracts and also in gastric and intestinal juices and was shown to have a molecular mass of 66 kDa, estimated by gel filtration. Differences in the molecular data for IF found in different parts of the gastrointestinal tract were considered to be insignificant, and it was suggested that the much higher proportion of IF to R-binder found in intestinal juice compared with unstimulated gastric juice could be due to the degradation of R-binder by pancreatic enzymes. These observations have been extended in the present study, which has identified the canine pancreas as an important source of an IF that closely resembles gastric IF in the dog.

In humans, pentagastrin causes release of IF stored in tubovesicles of gastric parietal cells (20, 35). Secretion increases relatively rapidly, peaks before gastric acid output, then declines quickly (33, 35). Similarly, the present study indicates that pentagastrin stimulates the rapid secretion of gastric IF in the dog, consistent with release of preformed protein. However, there may be important differences in the secretion of gastric Cbl-binding proteins comparing the dog to humans, since preliminary immunohistochemical findings, using the antiserum to canine gastric IF from the present study, suggest that the cellular origin of gastric IF in the dog may be peptic rather than parietal cells (34). In addition, while the stomach appears to be a relatively poor source of R-binder in humans (18, 21), stimulated outputs of gastric IF and R-binder were in equal proportions in the present study and profiles approximated one another; however, it is not clear whether this finding reflects similarities in the secretory mechanism or a common cellular origin for these Cbl-binding proteins in the dog. While gastric IF has been localized to parietal cells in many species, this protein has been found in peptic cells in the mouse and rat, and isolated peptic cells from the latter have been shown to secrete IF in parallel with pepsinogen (12). Gastrin and pentagastrin can stimulate pepsinogen secretion in intact animals, but study of canine peptic cells in monolayer culture suggests that direct action of gastrin is not the physiological mechanism (27). The cellular localization and physiological mechanism for stimulation of gastric IF secretion in the dog are therefore unclear, and consequently there needs to be some caution in direct comparison of the stimulated outputs of IF from the stomach and pancreas in the present study, since these may be different after a meal. Nevertheless, the findings clearly show that the pancreas is a major source of this Cbl-binding protein in the dog and provide the first conclusive evidence for the secretion of a pancreatic IF in any species.

That the canine pancreas might be a source of IF was suggested by finding cross-reactivity between pancreatic extracts and an antiserum raised to gastric Cbl-binder (1). This possibility was supported by a further brief report that indicated that malabsorption of Cbl in gastrectomized dogs with ligated pancreatic ducts could be reversed by administration of an extract of canine pancreas with trypsin (2). Malabsorption of Cbl in these dogs was unlikely to be solely due to defective proteolytic degradation of R-binders, since trypsin alone had no effect but could reverse Cbl malabsorption when duct ligation was not accompanied by gastrectomy. However, the Cbl-binding proteins in gastric and pancreatic secretions were not characterized. Indeed, the conclusion that tryptic digestion is needed for the activation of pro-IFs in the dog suggests that the potential contribution of R-binders to the Cbl-binding capacities of these secretions had been overlooked.

In the present study, mean concentrations of IF and also peak outputs per minute were approximately 5- to 10-fold higher in pancreatic juice after stimulation with

secretin and CCK, respectively, than in pentagastrin-stimulated gastric juice. These data indicate that the pancreas can make a major contribution to the total output of IF in the dog, although there may be differences in the relative outputs of IF from these two sites during physiological stimulation with a meal, as discussed above. The parallel outputs of IF and trypsinogen after secretin administration could repesent a washout phenomenon, as suggested for pancreatic secretory products in humans (26) but may also reflect contamination of the secretin preparation with CCK. Consequently it is not certain whether secretin can specifically stimulate secretion of canine pancreatic IF. However, stimulation by CCK is indicated by the increased peak outputs of IF and of trypsinogen, but not of fluid, in response to subsequent administration of this secretagogue. Preliminary immunohistochemical studies, using the antiserum to canine gastric IF shown here to cross-react with pancreatic IF, suggest that duct cells are the source of pancreatic IF in the dog (34), but the implication that CCK may stimulate secretion of IF by pancreatic duct cells, either directly or indirectly, has yet to be confirmed.

Canine gastric and pancreatic IFs were purified by use of Cbl-Sepharose, an affinity matrix that has been used previously for the purification of Cbl-binding proteins in other species (3, 4, 16, 31). Non-IF Cbl-binding proteins were prevented from adsorbing to the affinity matrix by cobinamide, a Cbl analogue that has a high affinity for R-binders but little affinity for IF (22, 24). Successful purification and separation of IF from R-binders was demonstrated by assaying Cbl-binding capacities of purified gastric and pancreatic IFs and showing lack of cobinamide inhibition, by gel filtration, which yielded single symmetrical peaks of apparent molecular mass 65 kDa, in agreement with previous findings for gel filtration of canine gastric IF (24), and by SDS-PAGE, which revealed single bands corresponding to a molecular mass of ~53 kDa. Gel filtration and SDS-PAGE can give falsely high estimates of the molecular mass of a glycoprotein, and this problem is relevant to determinations of molecular mass of IF in other species (12). For example, the molecular mass of human gastric IF has been estimated as 66 and 59 kDa by these two procedures, respectively, whereas sedimentation equilibrium ultracentrifugation gave an estimate close to the true molecular mass of 44 kDa determined by amino acid and carbohydrate analyses (3). In the present study, values approximately comparable to those for human IF were derived by gel filtration and SDS-PAGE of gastric and pancreatic IFs; consequently, their true molecular mass may also be similar to that of the human protein.

In addition to an identical molecular mass, similarities between canine gastric and pancreatic IFs were further emphasized by demonstration of immunologic cross-reactivity and comparable association constants for receptors in ileal brush-border vesicles. The latter indicated a strong affinity of the purified IFs for ileal receptors, and the values are similar to those observed previously (24) for binding of human intrinsic factor to dog ileal receptors. There was greater binding of the IF-cyano[^{57}Co]Cbl complexes to brush-border vesicles prepared from ileum than from jejunum, confirming the specificity of receptor-binding sites of both purified proteins. In addition, dissociation from ileal vesicles after incubation with EDTA was compatible with release of IF-cyano[^{57}Co]Cbl complexes from receptor protein in the microvillar membrane (23).

This study therefore demonstrates that the canine pancreas is an important source of an intrinsic factor that closely resembles gastric intrinsic factor in the dog. Further experiments indicate that these purified proteins can promote ileal uptake of Cbl by receptor-mediated endocytosis, suggesting that both gastric and pancreatic IFs could play a physiological role in the absorption of Cbl in the dog (6).

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